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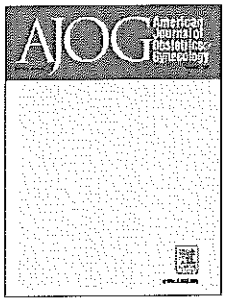

Host Response to Synthetic Mesh in Women with Mesh Complications

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HOST RESPONSE TO SYNTHETIC MESH IN WOMEN WITH MESH COMPLICATIONS

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DISCLOSURE
Moalli: cooperative research agreement ACell
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35 CONDENSATION

36 A persistent macrophage response was observed in mesh tissue complexes excised from women
37 with mesh complications months to years following implantation, with differences observed in
38 biochemical markers for exposure vs pain.

39 SHORT VERSION OF TITLE

40 Vaginal macrophage profile in women with mesh complications.

41

ABSTRACT

Background: In spite of good anatomical and functional outcomes, urogynecologic polypropylene meshes used to treat pelvic organ prolapse and stress urinary incontinence are associated with significant complications, most commonly mesh exposure and pain. Few studies have been performed that specifically focus on the host response to urogynecologic meshes. The macrophage has long been known to be the key cell type mediating the foreign body response.

Conceptually, macrophages responding to a foreign body can be broadly dichotomized into M1 pro-inflammatory and M2 pro-remodeling subtypes. A prolonged M1 response is thought to result in chronic inflammation and the formation of foreign body giant cells with potential for ongoing tissue damage and destruction. While a limited M2 predominant response is favorable for tissue integration and ingrowth, excessive M2 activity can lead to accelerated fibrillar matrix deposition, resulting in fibrosis and encapsulation of the mesh.

Objectives: To define and compare the macrophage response in patients undergoing a mesh excision surgery for the indication of pain versus a mesh exposure.

Study design: Patients scheduled to undergo a surgical excision of mesh for pain or exposure at Magee-Womens Hospital were offered enrollment. Twenty-seven mesh-vagina complexes removed for the primary complaint of a mesh exposure (N=15) vs pain in the absence of an exposure (n=12) were compared to 30 full thickness vaginal biopsies from women undergoing benign gynecologic surgery without mesh. Macrophage M1 pro-inflammatory versus M2 pro-remodeling phenotypes were examined via immunofluorescent labeling for cell surface markers CD86 (M1) vs CD206 (M2) and M1 vs M2 cytokines via enzyme-linked immunosorbent assay (ELISA). The amount of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9

(MMP-9) proteolytic enzymes were quantified by zymography and substrate degradation assays, as an indication of tissue matrix degradation. Statistics were performed using one-way ANOVA with appropriate post hoc tests, t-tests, and Fisher's Exact test.

Results: Twenty-seven mesh-vaginal tissue complexes were excised from 27 different women with mesh complications - 15 incontinence midurethral slings and 12 prolapse meshes. Upon histological examination, macrophages surrounded each mesh fiber in both groups, with predominance of the M1 subtype. M1 and M2 cytokines/chemokines, MMP-9 (pro and active), and MMP-2 (active) were significantly increased in mesh-vagina explants as compared to vagina without mesh. Mesh explants removed for exposure had 88.4% higher pro-MMP-9 ($p=0.035$) than those removed for pain. A positive correlation was observed between the profibrotic cytokine IL-10 and the percentage of M2 cells ($r=0.697$, $p=0.037$) in the pain group.

Conclusion: In women with complications, mesh induces a pro-inflammatory response that persists years after implantation. The increase in MMP-9 in mesh explants removed for exposure indicates degradation while the positive association between IL-10 and M2 macrophages in mesh explants removed for pain is consistent with fibrosis.

Keywords: cytokines, inflammatory response, macrophage phenotype, polypropylene mesh

INTRODUCTION

Over the past decade, lightweight, wide-pore polypropylene mesh has been increasingly used in the repair of pelvic organ prolapse and stress urinary incontinence. In spite of favorable anatomical and functional outcomes, mesh use has been associated with complications, most commonly mesh exposure through the vaginal epithelium and pain.[1] Studies of similar meshes used in hernia repair have demonstrated that all polypropylene meshes induce a prolonged inflammatory response at the site of implantation.[2-3] The magnitude and type of response is associated with the development of complications.[3-4] Although it is tempting to extrapolate these findings to meshes applied to the vagina, data suggests that the host response at these two sites is distinct. [5]

The host response following the placement of any foreign material into the body has been well described. Following the initial recruitment of neutrophils, macrophages become the primary immune cell involved in the clearance of debris and the initiation of the host response.[6-7] While this is an essential initial component of healing, the long term presence of activated inflammatory cells, such as macrophages at the mesh tissue interface, can negatively impact the ability of the mesh to function as intended.

Macrophages have been classified as having diverse and plastic phenotypes along a continuum between M1 (classically activated; pro-inflammatory) and M2 (alternatively activated; remodeling, homeostatic) extremes.[8-10] M1 macrophages are characterized by the secretion of reactive oxygen species, pro-inflammatory cytokines and chemokines, and can be identified via the cell surface marker CD86. Persistence of M1 macrophages can lead to tissue damage and destruction. In contrast, M2 macrophages secrete growth factors and anti-inflammatory immune modulators and can be identified by the cell surface marker CD206. M2

105 macrophages participate in the constructive healing and remodeling phase of the foreign body
106 response resulting in tissue deposition and in growth.[8, 10-12] However, an overzealous M2
107 response can also lead to excess tissue deposition and fibrosis.[13] As such, macrophage
108 polarization and plasticity play an important and determinant role in tissue remodeling following
109 injury and the integration of biomaterials. [13-14] Limited data exists on the macrophage response
110 following implantation of urogynecologic meshes[15-16] particularly in regard to implantation in
111 the vagina.

112 The objective of the current study was to characterize the macrophage response present in
113 patients undergoing mesh excision surgery and to define differences in this response according to
114 the two most common complications - mesh exposure and pain[1] Mesh-tissue constructs from
115 women undergoing mesh excision surgery for the indication of pain and exposure were
116 compared to full thickness vaginal biopsies from women undergoing prolapse repairs using
117 morphological, biochemical, and immunological endpoints.

118

119 **MATERIALS AND METHODS**

120 **Patient Acquisition**

121 Patients scheduled to undergo surgical excision of mesh as part of a larger study (Magee
122 Mesh Biorepository IRB# 10090194) were offered enrollment. For inclusion in the current
123 study, mesh had to be removed from the anterior or apical compartment for the primary
124 indication of exposure or pain. Mesh exposure was defined as at least 2 mm of mesh visible
125 through the vaginal epithelium and pain was defined as mesh being removed for the primary
126 complaint of pain (with palpation, ambulation, or intercourse) without evidence of exposure.
127 Patients were excluded from the study if presenting with acute infection (fever, worsening pain,

and pus in area of mesh) or erosion into the bowel or bladder. Patients were also excluded if they were unable to provide informed consent, on chronic immunosuppressive therapy, or had an autoimmune disorder. After consent was obtained, baseline demographic data were abstracted from the electronic medical record including age, race/ethnicity, body mass index (BMI), gravidity, parity, hormone use, menopausal status, and smoking status (Table 1). Menopausal status was defined as: pre-menopausal (regular menstrual periods within the last 12 months) and post-menopausal (no menstrual periods within the last 12 months). Hormone use was defined as current use of systemic estrogen with or without progesterone or vaginal estrogen for $\geq$ to 3 months. Smoking was defined as current smoker (yes/no). Operative reports from the initial mesh surgery were reviewed and the type of mesh recorded

On the day of surgery, the excised mesh-tissue complex was placed in a sterile specimen container, immediately placed on ice, and sent for analysis. Samples from mesh patients were age, BMI, and menopausal status-matched to full thickness vaginal biopsies obtained from the anterior vagina at the vaginal apex in mesh-naïve women with stage II or III prolapse with and without incontinence undergoing pelvic surgery, as described previously (IRB # 0412054).[17] This group of vaginal biopsies was selected as a control for the present study as there is evidence that the vaginal biopsies from women with prolapse and incontinence have increased MMPs relative to women with normal pelvic organ support. [18-19]

Tissue Extract Acquisition and Histological Preparation

Excised tissue-mesh complexes and non-mesh control tissues were extracted in high salt extraction buffer as described previously.[20] Additional pieces of vagina-mesh-complexes and

non-mesh control tissue were embedded into O.C.T. compound (Tissue-Tek®, Sakura Finetek USA. Inc., Torrance, CA), flash frozen in liquid nitrogen, sectioned (7μm) and stored at -80°C.

Cytokine and Chemokine Determination

Quantification of cytokines IL-10, IL-4, TNF-α, IL-12p70, and IL-12p40p70 and chemokines CXCL10 and CCL17 was performed using commercially available ELISA kits (Life Technologies, Carlsbad, CA and R&D Systems, Minneapolis, MN, respectively). All samples were run in duplicate or triplicate using 40ug total protein per sample per assay. A patient sample that had been previously characterized for analyte amounts served as an internal control.

Zymographic Analyses

Samples containing 30μg total protein were analyzed in duplicate by substrate zymography according to manufacturer's instructions (Novex, Life Technologies, Carlsbad, CA). For quantification of active and pro-enzyme forms of MMP-2, band density was measured with ImageJ Software (National Institute of Health, Bethesda, MD), and measurements were normalized to an internal control.

MMP-9 activity assay

Endogenous MMP-9 activity was measured using a Fluorokine E Human Active MMP-9 kit (R&D systems, Minneapolis, MN). For each experiment, 20 μg of protein from each sample was tested in duplicate. Fluorescence was read using a Spectramax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 320 nm and an emission wavelength of 405 nm. Data was analyzed using a four-parameter regression curve (Masterplex ReaderFit, Miraibio, San Francisco, CA) and normalized to nanogram active MMP-9 per mg protein in each sample.

Immunofluorescent Labeling

Tissue sections were quadruple-labeled for pan-macrophage marker CD68, M1 macrophage marker CD86, M2 macrophage marker CD206, and nuclear marker 4',6-diamidino-2-phenylindole (DAPI) as described[15] and imaged using a Nikon ECLIPSE 90i upright microscope (Nikon USA, Melville, NY). For mesh-vagina complexes, six 200X images were acquired over two locations within the tissue. Three images were taken in an area of a mesh fiber (defined as a single fiber of polypropylene not immediately adjacent to other fibers) and three additional images were taken in the area of a mesh knot (defined as three or more single fibers immediately adjacent to each other). For control patients, six 200X images were taken over two areas of each tissue. For each image, two trained technicians counted the number of total cells and the number of cells co-expressing either CD68 and CD86 or CD68 and CD206 to define the M1 and M2 macrophage population, respectively. For each field the M2:M1 ratio was calculated as described in Wolf et al [21] by using the formula (raw number of M2 macrophages+1)/(raw number of M1 macrophages +1) to avoid division by 0 in samples with no cells present.

Statistical Analysis

Power analysis showed 8 samples in each group were necessary to reach statistical significance for cytokines using previously obtained IL-10 values in vaginal extracts from non-human primate with and without Gynemesh PS implanted via sacrocolpopexy. [15] Statistical analysis was performed with SPSS 21 (IBM, Armonk, NY). For demographic data, a one-way ANOVA with Tukey *post hoc* testing, Kolmogorov-Smirnov tests, and Fisher's Exact tests with significance level α=0.05 was used. For the biochemical endpoints of mesh patients vs. control patients and for the complication exposure vs. pain, a two-tailed Student's independent samples t-test was performed. For histological endpoints comparing areas of a mesh fiber to a mesh knot,

a paired-sample t-test was used (α=0.05), and when comparing tissue removed for exposure vs. pain, an independent-sample t-test was used (α=0.05). Pearson's correlations were used to correlate cytokines with histologic findings.

RESULTS

Demographic Data

Twenty-seven mesh-vaginal tissue complexes were excised from 27 women - 15 incontinence midurethral slings and 12 prolapse meshes. Four of the 27 meshes were implanted via abdominal sacrocolpopexy and the remainder were inserted transvaginally. There were no differences in patient age, race/ethnicity, BMI, gravidity, parity, hormone use, menopausal status, smoking status, or duration of mesh implantation (all $p>0.05$, Table 1). In addition, there were no differences in patient demographics or experimental endpoints when separated by prolapse meshes vs midurethral slings. The specific meshes that were excised are listed in Table 2. Mesh type was not found in 3 meshes removed for pain and 1 mesh removed for exposure. Meshes were explanted from 4.5 to 93 months following the index surgery.

Biochemical Endpoints

All M1 pro-inflammatory and M2 pro-remodeling cytokines and chemokines were increased in mesh explants as compared to non-mesh tissue (Table 3), indicating a robust, active and ongoing host response to polypropylene long after implantation. Examination of the pro-remodeling cytokines IL-4 and IL-10 showed a 1.18 fold increase ($p=0.011$) and 1.45 fold increase ($p=0.016$), respectively, in mesh-vagina explants vs. control. Pro-inflammatory cytokines TNF-α, IL-12p40p70, and IL-12p70 had a 2.13 fold increase ($p<0.001$), a 2.19 fold increase ($p<0.001$), and a 1.22 fold increase ($p=0.001$), respectively, in mesh explants vs.

controls. The pro-inflammatory chemokine CXCL10 and the pro-remodeling chemokine CCL17 were 3.38 fold increased ($p=0.016$) and 7.26 fold increased ($p<0.001$) relative to control, respectively. Comparison of the ratio of the M2 pro-remodeling cytokines (IL-10 + IL-4) to the M1 pro-inflammatory cytokines (TNF-α + IL-12p70) revealed a decrease in mesh explants as compared to controls ($p=0.003$), indicating a shift towards a pro-inflammatory profile.

Pro- and active MMP-9 were 2.85 fold increased ($p<0.0001$) and 2.91 fold increased ($p<0.0001$) respectively, in mesh explants as compared to controls. While proMMP-2 was similar in complexes with and without mesh, active MMP-2 was 2.08 fold increased ($p=0.038$).

Pro-MMP-9 was 1.88 fold higher with exposure than pain ($p=0.036$). No statistical difference was observed for active MMP-9 ($p=0.067$). Values for individual cytokines and chemokines and for levels of pro- and active MMP2 did not differ based on indication for mesh removal (Table 4).

Immunofluorescent Labeling

Mesh tissue complexes demonstrated a marked but highly localized foreign body response characterized by the presence of CD68+ cells (macrophages) surrounding each mesh fiber. In areas where mesh fibers were in close proximity, the host response to neighboring fibers overlapped increasing the magnitude of the response. Labeling with the M1-specific surface marker CD86 and the M2-specific marker CD206 demonstrated that control tissues contained few or no macrophages, as opposed to tissue-mesh-complexes in which CD68+CD86+ pro-inflammatory M1 macrophages were concentrated around mesh fibers (Figure 1, Table 5). Mesh explants contained a higher number of total cells/200X field when compared to controls (682.46

± 142.61 vs 441.63 ± 126.13 cells, p<0.001) and a lower ratio of M2:M1 macrophages (0.260 ± 0.161 vs 1.772 ± 1.919, p=0.001), supporting an ongoing pro-inflammatory response.

In mesh explants, no differences in the number of macrophages present or the macrophage phenotype was observed when data was stratified based on indication for mesh removal (Table 6). However, when comparing areas containing a mesh fiber vs. a mesh knot in individual samples, the area of a mesh knot had a total cell density that was 1.23 fold increased (p=0.037). Within the area of a mesh knot, the number of M1 macrophages was 2.00 fold increased (p=0.003), the percentage of M1 macrophages (as a function of total cells) was 1.59 fold increased (p=0.004), the number of M2 cells was 2.27 fold increased (p<0.001), and percentage of M2 macrophages was 1.77 fold increased (p=0.002) as compared to a single mesh fiber (Table 7) suggesting that the host response was proportional to the amount of material in contact with the host. No significant difference was observed in the ratio of M2:M1 macrophages in the area of a mesh knot vs a mesh fiber. A positive correlation between IL-10 and the percentage of pro-remodeling/pro-fibrotic M2 cells (r=0.697, p=0.037) in the pain group was observed that was not present in the exposure group. In the exposure group, a positive correlation was observed between the pro-inflammatory cytokine IL-12p40p70 and percentage of pro-inflammatory M1 macrophages (r=0.584, p=0.059), which did not reach statistical significance. This correlation was not observed in the pain group.

COMMENT

A persistent foreign body response was observed in mesh tissue complexes excised from women requiring surgical excision of mesh months to years after mesh implantation. The host response was characterized by a predominance of macrophages with an increase in both pro-

inflammatory and pro-remodeling cytokines/chemokines along with increased tissue degradation as evidenced by increased MMP-2 and MMP-9. Mesh-tissue complexes removed for mesh exposure had increased pro-MMP-9 indicating a pro-inflammatory and tissue destruction type response. In contrast, in mesh-tissue complexes removed for pain, the percentage of M2 macrophages (involved in tissue remodeling and fibrosis [22-24]) positively correlated with the amount of the anti-inflammatory/pro-fibrotic cytokine IL-10 consistent with tissue deposition and encapsulation.

The presence of macrophages, elevated cytokines, chemokines and MMPs in tissue-mesh complexes excised from patients with exposure or pain suggests that polypropylene mesh elicits an ongoing host inflammatory response.[7] Importantly, the presence of macrophages was limited to the area immediately surrounding the mesh fibers with each fiber eliciting an independent reaction, the magnitude of which appeared to be proportional to the number of fibers in a given area. This points to the importance of maintaining meshes in a flat (as opposed to folded) configuration to minimize the amount of material per area and choosing meshes in which the spaces between fibers (pores) are wide enough that the host response to two adjacent fibers does not overlap.[25]

In mesh-tissue complexes removed for exposure, pro-MMP9 was increased as compared to mesh removed for pain, suggesting a degenerative process. Studies of hernia meshes in animal models have shown that persistent inflammation with a prolonged release/activation of MMPs can lead to the degeneration of mesh-implanted tissues resulting in a deterioration in structural and mechanical integrity.[26-27] In a nonhuman primate model, Gynemesh PS caused a decrease in key structural proteins (collagen and elastin) and increased MMP activity leading to thinning of the underlying and associated tissues and a deterioration of mechanical properties.[20, 28-29]

283 Gynemesh PS has a highly unstable geometry when loaded resulting in pore collapse and
284 increasing stiffness of the product. [30-31] As virtually all meshes removed from women with
285 complications have evidence of deformation and pore collapse, [32-36] mesh exposure may
286 represent a mechanical phenomenon in which altered mesh geometries result in increased mesh
287 stiffness which in turn, leads to a degenerative response as a result of stress shielding and
288 ongoing destructive inflammation.

289 While the mechanism for developing pain following mesh implantation is not clear, mesh
290 deformation (contraction, retraction, or shrinkage) is also frequently observed in meshes
291 removed for pain. [32, 34, 36-38] In normal wound healing, as inflammation resolves and remodeling
292 begins, some amount of tissue contraction occurs with the formation of a scar.[38] In the presence
293 of a permanent foreign body, the implant is surrounded with a fibrotic capsule since it cannot be
294 degraded. For hernia meshes, if the fibers are too close (<1mm), the fibrotic response to
295 neighboring fibers overlaps, or "bridges," resulting in "bridging fibrosis" or encapsulation of the
296 mesh.[25] As myofibroblasts constitute the primary cellular component of the fibrotic capsule,
297 when a mesh becomes encapsulated, the resulting contraction or "shrinkage" may place tension
298 on adjacent tissues resulting in pain. Indeed, mesh shrinkage (50-70%) has been described to
299 occur following transvaginal insertion of prolapse meshes.[32-37] In the present study, in meshes
300 removed for pain, IL-10, a cytokine that, in increased amounts, has been associated with
301 fibrosis,[39-42] positively correlated with the percentage of M2 polarized macrophages
302 (remodeling/fibrotic phenotype), supporting an ongoing remodeling/fibrotic process involved in
303 at least one mechanism leading to pain.

304 A major limitation of the current study is that it was not possible to include a control group
305 of mesh-tissue complexes obtained from women who underwent mesh implantation without a

306 complication. As such, the current study does not assess the inflammatory response to prolapse
307 mesh in women with a good outcome and focuses only upon the inflammatory response in the
308 setting of complications. Our strict inclusion criteria resulted in a limited sample size which
309 limits the generalizability of the data especially in regards to the impact of variables such as age,
310 length of time of mesh implantation, and hormone use. All patients enrolled in the current study
311 completed the Pelvic Pain Scale (PPS) and a number of other validated questionnaires, the
312 analysis of which is pending and will be the focus of a future manuscript. Finally, it is our
313 practice to remove as much mesh as possible in patients presenting with a mesh complication;
314 therefore, we cannot guarantee that the mesh undergoing analysis was at the exact site of the
315 complication, regardless of whether it was removed for an exposure or pain.

316 In conclusion, the findings of present study suggest that the two major mesh
317 complications - exposure and pain - are associated with a marked pro-inflammatory response that
318 persists years after mesh implantation. In addition, different mechanisms for mesh exposure and
319 pain may be associated with differential macrophage activation. Future studies will focus on the
320 specific risk factors that predispose to specific types of mesh complications.

321 **AUTHOR CONTRIBUTIONS**

322 A.L.N. acquiring samples, conducting experiments, writing the manuscript, and analyzing data;
323 B.B. analyzing data and writing the manuscript; R.L acquiring data, analyzing data, and writing
324 the manuscript; S.P acquiring samples, and conducting experiments; M.B. acquiring data and
325 analyzing data; S.A. acquiring data and analyzing data; P.A.M. designing research studies,
326 analyzing data, and writing the manuscript.

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329

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436 **Table 1.** Descriptive statistics of study population.

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	Mesh complications (n=27)			
	Mesh exposure (n=15)	Pain (n=12)	Prolapse (n=30)	P-value
Age[a] (years)	56.1 ± 8.0	52.1 ± 9.7	52.9 ± 9.2	0.527
BMI[a]	30.8 ± 5.9	27.3 ± 3.3	28.5 ± 4.0	0.158
Gravidity[b]	3.0 (2.0, 4.0)	3.5 (2.0, 5.75)	2.0 (2.0, 3.0)	0.644
Parity[b]	2.0 (2.0, 3.0)	3.0 (2.0, 5.0)	2.0 (2.0, 3.0)	0.899
Time implanted (months)[a]	36.9 ± 30.3	30.9 ± 18.0	NA	0.527
Menopausal status[c]				
pre-menopausal	2(13%)	3(25%)	12(40%)	
post-menopausal	13(87%)	9(75%)	18(60%)	0.190
Smoking[c]				
nonsmoker	12(80%)	9(75%)	27(90%)	
smoker	3(20%)	3(25%)	3(10%)	0.713
Race/ethnicity[c]				
white	15(100%)	12(100%)	29(97%)	
Indian	0	0	1(3%)	
other	0	0	0	1.000
Hormonal Usage[c]				
Yes	7(47%)	7(58%)	7(23%)	
No	8(53%)	5(42%)	23(77%)	0.077

438 a. values given as mean ± standard deviation
439 b. values given as median (25 percentile, 75 percentile)
440 c. values given as number of patients (percentage)
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447 **Table 2**. Excised mesh brand and type categorized by mesh complications. Of the 27 patients
448 enrolled in this study, mesh brand information was not able to be determined for 4 of the
449 patients.

Mesh device	Removal due to exposure (n=15)	Removal due to pain (n=12)
AMS Monarc TOT	1	0
AMS Perigee	1	0
Bard Ajust Single Incision Sling (SIS)	0	1
Bard Soft Mesh	0	1
Boston Scientific Lynx TVT	1	1
Boston Scientific Obtryx TOT	1	0
Boston Scientific Solyx mini sling	0	1
Boston Scientific Uphold	1	0
Caldera Desara Sling System for SUI	1	0
Coloplast Novasilk	1	0
Gynecare Prolift mesh kit	1	1
Gynecare Gynemesh PS	4	0
Gynecare TVT Secur	1	2
Gynecare TVT	1	1
Gynecare TOT	0	1
Original medical records not available	1	3

Table 3. Distribution of Biomarkers by Study Group.

	Mesh complications (exposure + pain) (n=27)	Prolapse control (n=30)	P-Value
Cytokines & Chemokines*			
IL-4	3.316 ± 0.860	2.800 ± 0.567	0.011
IL-10	17.738 ± 9.957	12.218 ± 5.932	0.016
TNF-α	15.963 ± 6.392	7.487 ± 2.224	0.000
IL-12p40p70	28.519 ± 16.437	13.029 ± 7.227	0.000
IL-12p70	2.070 ± 0.471	1.691 ± 0.291	0.001
CXCL10	62.534 ± 71.835	18.492 ± 61.065	0.016
CCL17	33.940 ± 38.156	4.674 ± 3.681	0.000
Proteases			
Pro-MMP-2$^{\perp}$	1.192 ± 0.851	1.324 ± 0.971	0.601
Active MMP-2$^{\perp}$	1.521 ± 1.636	0.733 ± 0.783	0.038
Pro-MMP-9 $^{\gamma}$	3.496 ± 2.643	1.226 ± 1.715	0.000
M2/M1 cytokines & chemokines			
(IL-4+IL-10)/ (TNF-α +IL-12p70)	1.209 ± 0.562	1.633 ± 0.486	0.003
CCL17/CXCL10	0.852 ± 0.921	0.661 ± 0.597	0.364

Data expressed as Mean ± Standard Deviation

*pg per 40μg protein, $^{\perp}$arbitrary units, $^{\gamma}$ng per 15μg total protein

453 **Table 4.** Comparison of the profiles of cytokines, chemokines and MMP2 in vaginal tissue
454 excised for mesh exposure and pain.

	Mesh: Exposure	Mesh: Pain	P-Value
Cytokines & Chemokines*			
IL-4	3.307 ± 0.973	3.328 ± 0.736	0.952
IL-10	19. 323 ± 11.665	15.757 ± 7.308	0.342
TNF-α	15.954 ± 5.011	15.976 ± 8.039	0.994
IL-12p40p70	31.739 ± 20.242	24.493 ± 9.235	0.231
IL-12p70	2.040 ± 0.564	2.108 ± 0.342	0.705
CXCL10	71.384 ± 86.873	51.472 ± 48.495	0.459
CCL17	38.445 ± 49.238	28.312 ± 17.132	0.467
Proteases			
Pro-MMP-2$^{\perp}$	0.965 ± 1.018	1.420 ± 0.606	0.200
Active MMP-2$^{\perp}$	1.278 ± 0.887	1.764 ± 2.164	0.483
Pro-MMP-9$^{\gamma}$	4.860 ± 3.464	2.586 ± 1.660	0.036
Active MMP-9$^{\gamma}$	6.063 ± 4.674	3.578 ± 1.338	0.067
M2/M1 cytokines & chemokines			
(IL-4+IL-10)/ (TNF-α +IL-12p70)	1.266 ± 0.640	1.138 ± 0.462	0.551
CCL17/CXCL10	0.864 ± 1.165	0.837 ± 0.529	0.937

455 Data expressed as Mean ± Standard Deviation

456 *pg per 40µg protein, $^{\perp}$arbitrary units, $^{\gamma}$ng per 15µg total protein

457 **Table 5.** Macrophage phenotypes in vaginal tissue excised for mesh complications as compared
458 to tissue derived from prolapse women.
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	Mesh complications (n=20)	Prolapse (n=24)	P-value
Total Nuclei	682.46 ± 142.61	441.63 ± 126.13	0.000
M1 cell count	197.95 ± 97.15	0.889 ± 2.40	0.000
% M1 cells	28.06 ± 11.03	0.181 ± 0.469	0.000
M2 cell count	41.90 ± 20.33	1.067 ± 2.034	0.000
% M2 cells	6.15 ± 2.80	0.313 ± 0.756	0.000
M2/M1 ratio	0.260 ± 0.161	1.772 ± 1.919	0.001

460 Data expressed as Mean ± Standard Deviation
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469 **Table 6.** Comparison of macrophage phenotypic profile in vaginal tissue excised for mesh
470 exposure and pain.

	Exposure (n=11)	Pain (n=9)	P-Value
Total Nuclei	660.242 ± 130.892	709.615 ± 159.307	0.467
M1 cell count	167.008 ± 87.598	235.763 ± 99.497	0.124
% M1 cells	24.554 ± 10.669	32.344 ± 10.432	0.118
M2 cell count	40.296 ± 21.046	43.867 ± 20.494	0.706
% M2 cells	6.139 ± 3.157	6.164 ± 2.474	0.984
M2/M1 ratio	0.301 ± 0.206	0.210 ± 0.061	0.189

471 Data expressed as Mean ± Standard Deviation

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473 **Table 7.** Comparison of macrophage phenotypic profile in areas of mesh knots and fibers.

	Mesh knot (n=13)	Mesh fiber (n=13)	P-Value
Total Nuclei	659.87 ± 133.57	536.77 ± 168.30	0.037
M1 cell count	197.79 ± 97.02	98.79 ± 58.79	0.003
% M1 cells	28.89 ± 11.63	18.13 ± 9.88	0.004
M2 cell count	47.08 ± 20.20	20.72 ± 13.24	<0.0001
% M2 cells	7.17 ± 2.83	4.06 ± 2.45	0.002
M2/M1 ratio	0.303 ± 0.186	0.274 ± 0.139	0.715

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FIGURE LEGENDS

Figure 1. Immunofluorescent labeling of pan-macrophage marker CD68 (red), M1 pro-inflammatory marker CD86 (orange), M2 pro-remodeling macrophage marker CD206 (green), and DAPI (blue). A, a mesh-tissue section from a patient presenting with an exposure and implanted with the AMS Perigee prolapse mesh for 93 months; B, a mesh-tissue section from a patient presenting with pain and implanted with the Gynecare TVT Secur for 6 months; C, control tissue from patients without graft implantation. A predominance of pro-inflammatory M1 macrophages surround mesh fibers (*) consistent with a prolonged immune response could be observed in both A and B; however, this response is limited to the area immediately adjacent to mesh fibers. Control tissue contained few or no macrophages as compared to mesh patient tissue. Magnification 200X.